

Neuropathology of Rett Syndrome

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ABSTRACT

Rett syndrome is a sporadic disorder (except for a few familial cases) occurring in 1 in 10,000 to 1 in 23,000 girls worldwide. It is associated with profound mental and motor handicap. About 90% of cases involve a mutation in the methyl-CpG binding protein 2 gene (*MECP2*). The role of this gene in the pathogenesis of this enigmatic disorder is being extensively investigated in animal models. Rett syndrome is associated with a complex phenotype that is unique in every aspect of its presentation, clinical physiology, chemistry, and pathology. Years of concentrated observations have defined the clinical presentation of classic Rett syndrome and its variants and related features (eg, neurophysiologic, radiologic, chemical, metabolic, and anatomic). This article reviews the neuropathology of Rett syndrome, which involves individual neurons, perhaps selected neurons, of decreased size, dendritic branching, and numbers of spines. This article also summarizes the studies in the human and mouse brain with Rett syndrome that are beginning to reveal the disorder's pathoetiology. (*J Child Neurol* 2005;20:747–753).

The features of the disorder that has become known as Rett syndrome were observed first in 1977 in Austria by Andres Rett¹ and in 1983 in Sweden by Hagberg and his European colleagues.² It is now recognized worldwide, usually presenting in a small girl with loss of speech and of purposeful hand use and the presence of stereotopies and ataxia. Some girls cannot walk, and many have epilepsy. Jellinger and Seitelberger made the first report of a series of eight Rett syndrome cases and observed decreased brain weight and decreased pigmentation of the substantia nigra pars compacta.³ Riederer and colleagues described the altered chemistry associated with Rett syndrome.⁴ Since these early reports, clinician-scientists have come to better understand this rare disorder in large part because the determination and encouragement of parents have prompted progress in the field. Even so, defining each aspect of the clinical-pathologic features of Rett syndrome has been difficult.

For example, it has been a challenge to determine that Rett syndrome is a genetic disorder. It affects girls and seems to be primarily a sporadic disease, with few familial cases. The identification of the X-linked *MECP2* as the causative gene required careful collaborations among laboratories, extensive study, and sharing of data.⁵ Mutations in the *MECP2* gene in exons 1 to 4 or DNA deletions have now been identified in more than 90% of Rett syndrome cases.^{5–8} Abnormalities in *MECP2* have also been detected in some boys with an infantile encephalopathy,⁹ in some children with an Angelman syndrome phenotype,¹⁰ and in some boys with mental retardation.¹¹ Three animal models are now being used to clarify the relationship of *MECP2* to the pathogenesis of Rett syndrome (Table 1).^{12–14}

The definition of the clinical features of Rett syndrome has also been evasive. Classic and variant forms have been defined. Genotype-phenotype correlations are now being made, and international data registries have been created to facilitate these associations.¹⁵

The patient with Rett syndrome is small, and studies of growth have identified disorders of swallowing mechanisms.¹⁶ The characterization of motor problems, absence of speech, loss of hand use, stereotopies, ataxia, dystonia, and hypertonicity or hypotonicity has been problematic.¹⁷ The cognitive aspects of Rett syndrome have evaded definition because of problems in testing patients who have no speech or hand use. Girls display abnormalities of breathing, heart rate, peripheral circulation, and emotional hyperexcitability that have to be distinguished from autonomic dysfunction and seizures.^{18–20} These complex clinical features in aggregate define a unique and demanding clinical situation requiring complete daily care by parents and innovative assistance from physicians and support groups. The relatively healthy patients with Rett syndrome survive into the fourth and fifth decades, although there is an increased incidence of sudden death in the adolescent years.²¹

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Table 1. Pathology of the Rett Syndrome Animal Models

Jaenisch models	
Germline <i>MECP2</i> deletion	
Generated using the Cre-loxP recombination system in which exon 3 (containing most of the methyl-CpG binding domain) is deleted	
Normal until 5 wk, fertile; develops nervousness, body trembling, pila erection, and occasional hard respiration	
Mice become overweight, deteriorate with weight loss at 8 wk and die by 10 wk	
Brains: reduced weight and neuronal size in hippocampus, cerebral cortex, and cerebellum	
Nestin-Cre-mediated <i>MECP2</i> deletion	
Resembles the germline deletion model	
Deleted the MeCP2 in postmitotic neurons, mainly in the postnatal forebrain, hippocampus, and brain stem; minimal in the cerebellum	
Mutants are healthy for 3 mo, when they became symptomatic, with a gain in weight, ataxic gait; survive up to 7.5 mo	
Brain pathology: decreased weight and neuronal size in cortex and hippocampus, not cerebellum	
Bird model: <i>MECP2</i> null	
Generated by replacing exons 3 and 4 of <i>MECP2</i> in ES cells: have deletions of all except the eight amino-terminal amino acids of MeCP2 protein	
Males and females appear normal initially	
Males at 3 to 8 wk develop stiff uncoordinated gait, hindlimb claspings, irregular breathing; teeth wear unevenly; testes are undescended	
Progression of symptoms, rapid weight loss; death at 543 d	
Females fertile; develop inertia and hindlimb claspings at 3 mo	
9 mo: breathing irregularity, decreased mobility; survive longer than males	
Zoghbi model: MeCP2^{308/y}	
Generated with a truncating <i>MECP2</i> mutation; using a stop codon after codon 308, the truncation retains the MBD, TRD, and nuclear localization signal	
Males appear normal for 6 wk and then develop tremors, motor impairment, hypoactivity, anxiety behavior, seizures, kyphosis, and stereotypic forelimb motions	
Survive to 1 yr: some die at 10 mo of unknown cause	
Mice are fertile	
Females survive longer with milder symptoms	

MBD = methyl-CpG binding domain; TRD = transcriptional repression domain.

NEUROCHEMISTRY

The neurochemistry of Rett syndrome (Table 2) has been difficult to determine in patients.²²⁻⁴¹ Studies on cerebrospinal fluid and brain tissue have analyzed transmitters, their receptors, and additional trophic factors. Abnormalities have been reported in most systems, including in acetylcholine, dopamine, serotonin, glutamate substance P, and new nerve growth factor. The age of the patient with Rett syndrome²⁹ and the severity of the symptoms³¹ influence measurements. Wenk concluded that the studies of neurotransmitters and peptides were variable except for acetylcholine, whose markers were consistently reduced.³⁵ Kaufmann concluded in his review that dopamine is involved in the striatonigral system and in forebrain choline acetyltransferase expression, with a moderate reduction in cortical cholinergic innervation and an elevation of glutamate and of some glutamate receptor subtypes in younger subjects with Rett syndrome.³² The clearer documentation of the alterations in chemistry with relation to age is still needed. The changes in the neurotransmitters and other trophic factors are of great importance, early on for their role as neurotrophins in brain development and later for their role in normal functioning of the mature brain. It is hoped that animal models will allow for more controlled examinations of how these important chemicals are altered in Rett syndrome.

PATHOLOGY

In this article, I elaborate on the observations that have been made suggesting that neuronal maturation is defective in Rett syndrome and on the observations about *MECP2* expression that coincide with these observations.

The infant girl with Rett syndrome is nondysmorphic, with a head circumference that is normal at birth but that begins to decelerate in its growth at 2 to 3 months of age. The other growth parameters (ie, height, weight, hand, foot size) decline later.⁴² Autopsies of patients with Rett syndrome of various ages reveal that all organs, except the adrenal gland, are small for the age of the patient but not for the height. The brain, however, is an exception. The average brain weight is 900 g, the brain weight of a 1-year-old infant. Thus, the weight of the Rett syndrome brain is significantly less than that of the brain of non-Rett syndrome controls for age and for height (Figure 1). The identification of this preferential involvement of the brain suggested that Rett syndrome was a disease of the nervous system. This has been endorsed by the observations that *MECP2* is expressed primarily in neurons.⁴³ The weight of the Rett syndrome brain does not decrease significantly with age, so atrophy does not account for the small size, an observation that has been confirmed by imaging studies of patients with Rett syndrome.³²

The decrease in brain weight is not generalized. The cerebral hemispheres are affected more than the cerebellum. The brainstem weights have not been compared with those of non-Rett syndrome brains because of the various techniques of brainstem dissection (Figure 2). Imaging studies define alterations in brain volumes in prefrontal, posterior frontal, and anterior temporal regions, with relative preservation in the posterior temporal and posterior occipital regions.^{44,45}

With routine examinations, the Rett syndrome brain reveals no obvious alteration in the blood vessels, cranial nerves, gyri, white matter, basal ganglia, cerebellum, brain stem, or spinal cord, although these have not been evaluated quantitatively in detail. Single reports of cerebellar and spinal cord neuronal loss with time

Table 2. Chemical Studies in Rett Syndrome

	Study
Biogenic amines	Riederer et al, 1985 ⁴
	Zoghbi et al, 1985, 1989 ^{22,23}
	Brucke et al, 1987 ²⁴
	Wenk et al, 1991, 1997, 1999 ²⁵⁻²⁷
	Perry et al, 1988 ²⁸
	Lekman et al, 1990 ²⁹
	Nielsen et al, 1992 ³⁰
	Percy, 1992 ³¹
	Kaufmann, 2001 ³²
	Budden et al, 1990 ³³
Opioids	Percy et al, 1994 ³⁴
Acetylcholine	Wenk, 1997 ³⁵
Glutamate	Hamberger et al, 1992 ³⁶
	Lappalainen and Riikonen, 1996 ³⁷
Nerve growth factor	Lappalainen and Vanhala, 1996 ³⁸
	Riikonen et al, 1999 ³⁹
Substance P	Matsuishi et al, 1997 ⁴⁰
	Deguchi et al, 2000 ⁴¹

have been recorded,^{46,47} and there have been no consistent reports of changes in glia or in the accumulation of lipofuscin. Kaufmann found some increase in glial fibrillary acidic protein expression but could not determine if this is a primary or a secondary phenomenon.³² It has been generally concluded that no recognizable degenerative, demyelinating, or gross malformative process explains the Rett syndrome phenotype.

STUDIES OF THE CEREBRAL CORTEX

To study the decreased size of the Rett syndrome brain, the cerebral cortex was examined with the Golgi technique. Pyramidal neurons of layers III and V of the frontal, motor, occipital, superior temporal, and inferior temporal cortices and layers II and IV of the subicular cortex and the neurons of Ca1 of the hippocampus were evaluated using camera lucida drawings and the Scholl analysis. There was a significant decrease in the dendritic territories in Rett syndrome of the frontal, motor, and subicular cortices when compared with non-Rett syndrome cases and trisomy 21 cases.^{48,49} The decreased dendritic territories in selected regions of

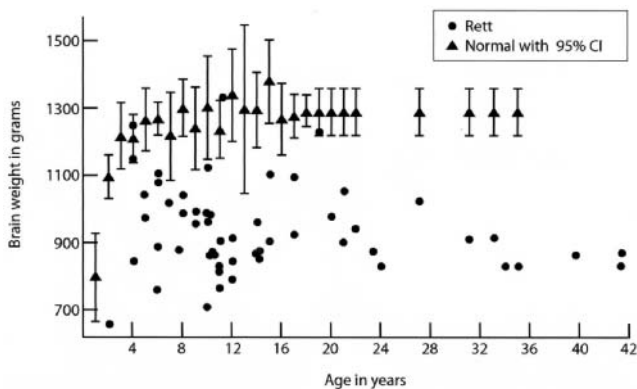


Figure 1. Weight of the Rett syndrome brain at autopsy. Note that there is no relationship between brain weight and age. CI = confidence interval.



Figure 2. The Rett syndrome brain is small, but with a normal external appearance. The cerebral hemispheres are relatively smaller than the cerebellum.

the Rett syndrome brain correlated with other observations that defined in the cortex, thalamus, basal ganglia, amygdala, and hippocampus a decreased neuronal size and in the hippocampus an increased neuronal packing density.⁵⁰ Casanova and colleagues studied cortical minicolumns in areas 9, 21, and 22 in five Rett syndrome brains. The minicolumns are reduced in size, significantly so, in area 21. The minicolumn is defined as the smallest functional unit of the cerebral cortex, and its diminished size in Rett syndrome could reflect the decreased dendritic branching of neurons that was observed in the Golgi studies or other undefined associations.⁵¹ Belichenko and colleagues made quantitative studies observing a decreased number of neurons in layers II and III of the frontal and temporal cortices⁵² and in the speech cortex a normal number of neurons, a preservation of the size differences between the dominant and nondominant hemispheres, and reduced numbers of parvalbumin-positive interneurons and synaptic sites.⁵³ Belichenko and colleagues observed decreased numbers of dendritic spines and afferent axons in the frontal cortex of four patients with Rett syndrome.^{54,55}

STUDIES IN OTHER BRAIN REGIONS

In the midbrain and substantia nigra, the large neurons of pars compacta have less melanin and tyrosine hydroxylase staining than controls.³ Imaging studies record decreased volumes of the midbrain, caudate, putamen, and thalamus.⁴⁴ The neurons of the globus pallidus were observed on Golgi studies to have altered contours and appendages in the region of the upper extremity.⁵⁶ In the cerebellum, a progressive loss of neurons with increasing age was observed in autopsy tissue⁴⁶ and by magnetic resonance imaging.⁵⁷ In the spinal cord, there is a report of anterior horn neuronal loss and gliosis in the corticospinal tracts of two patients.⁴⁷

The clinical expression of breathing irregularities, heart rate variability, small cold feet, constipation, and difficulties in swallowing suggests autonomic impairment. Functional studies of the vagus nerve define an abnormality of vagal tone.⁵⁸ Limited brainstem studies suggest abnormalities in serotonin receptors and in substance P content.^{41,59}

In the peripheral nerve, a mild distal axonal neuropathy was observed in 7 of 12 cases by Haas⁶⁰ and in 4 of Jellinger and colleagues' cases.⁶¹ Muscle biopsies have shown variable results, including type II atrophy, type I atrophy, myopathic changes (in a rare case), alterations in mitochondrial morphology, and a reduction in mitochondrial enzyme levels.⁶¹ Continuing reports of altered chemistry and mitochondrial enzymes suggest either primary or secondary involvement.⁶²⁻⁶⁴

In summary, the major morphologic change in the Rett syndrome brain is a decrease in the size of the brain and of individual neurons, which show less dendritic arborization and spines than the non-Rett syndrome neurons. There is no obvious atrophy or degeneration, so the neuropathologic process in Rett syndrome has been hypothesized to be one of a failure of development.

STUDIES RELATED TO MECP2

MECP2 modifies gene expression by inhibiting transcription, and this function would seem to be significant in the genetic control of brain development. The distribution of MeCP2 protein has been studied in the developing human brain and in Rett syndrome.⁶⁵ The earliest normal expression of MeCP2 at 10 gestational weeks is in the nuclei of isolated cells of the cerebral subventricular zone, of the brain stem, and of the subcortical and Cajal-Retzius neurons of the cerebral cortex. The expression of MeCP2 appears next in the thalamus, midbrain, and basal ganglia. The hippocampus and the cerebellum show less expression early in development, but with maturation, most neurons, but not glia, express MeCP2. The cerebral cortical neurons continue to show increasing amounts of MeCP2-immunopositive neurons into the adolescent years.⁴³

The interpretation of the immunochemical marking for MeCP2 has been complicated by the availability of consistent antibodies, by antibodies that identify various epitopes, and by the observed variability of intensity of staining at various ages and within the same types of neurons. Most staining appears in the nucleus, but there is slight cytoplasmic staining in some neurons whose cytoplasm can be recognized by routine histology. LaSalle and colleagues, using laser scanning cytometry, were able to quantitate the expression of MeCP2 in the human and murine brain and confirmed the presence of both a low and a high expression of MeCP2 immunoreactivity.⁶⁶ They studied the ontogeny of MeCP2 in the developing mouse brain and showed that near the time of birth (E10-E19), MeCP2 of the low expression type is abundant in the medulla and thalamus. The expression decreases after birth and then gradually increases (after 5 days) to increasing levels of high MeCP2 expression in all neurons in the brain (3-40 weeks).⁶⁷ The Ronnet laboratory showed a similar pattern of variable MeCP2 expression in the neurons of the olfactory epithelium and identified MeCP2 expression in the immature and mature olfactory receptor neurons prior to synaptogenesis in those neurons. They demonstrated in the mouse olfactory epithelium that when synaptogenesis was blocked by axotomy, the expression of MeCP2 was decreased.⁶⁸ These observations draw attention to the role of MeCP2 in synaptogenesis. Kaufmann's study on the location of MeCP2 revealed that it is present not only in the nucleus but also in the postsynaptic compartment of the neuron, where it is available to react in response to neuronal activity.⁶⁹ Ronnett's continuing examination of the effect of MeCP2 deficiency on the olfactory epithelium in the

MeCP2-null mouse⁷⁰ and in the olfactory epithelium of patients with Rett syndrome showed that their olfactory receptor neurons exhibit a delay in maturation. In addition, the axonal projections of the MeCP2-null animals are disorganized, resulting in a reduced size of the olfactory glomeruli.⁷¹

LaSalle studied the expression of MeCP2 in female mice heterozygous for MeCP2. The MeCP2-negative cells were found to be uniformly distributed, and using tissue microarrays, she observed that the percentage of MeCP2-negative cells was less than the expected 50%, in the range of 20% to 39%. This was interpreted to mean that an unbalanced X-chromosome inactivation pattern favors inactivation of the mutant allele. The MeCP2 expression pattern in the cells that do express MeCP2, however, differed from the Wt expression of MeCP2, exhibiting more cells of the low expression type and suggesting that mutant MeCP2 can influence the Rett syndrome phenotype by both cell-autonomous and non-cell-autonomous mechanisms. Similar observations were made on tissue microarrays of frontal cortex of three patients with Rett syndrome.⁶⁷

DISCUSSION OF MECP2 IN NEURONAL MATURATION AND IN RETT SYNDROME

In Rett syndrome, there is a low brain weight, a reduced branching of dendrites, and a decrease in dendritic spines. The studies on the ontogeny of MeCP2 expression reveal that MeCP2 is a marker of the "maturing neuron"^{43,72,73} being expressed in neurons as they mature and take up critical locations for the continuing development of the brain. The increase in MeCP2 expression in the brain after the prenatal period, when most apparent brain development occurs, suggests that MeCP2 might also be required for neuronal maintenance and function.

On the basis of these observations, and the fact that the child with Rett syndrome has a deceleration in growth of the head circumference during the time that rapid synaptogenesis is occurring, it has been postulated that synaptogenesis is deficient in Rett syndrome. This is supported by the observations that in mice MeCP2 is expressed prior to synaptogenesis so that, presumably, the MeCP2-deficient Rett syndrome brain would have fewer synapses. The animal models suggest a hypothesis that addresses the deficiency of synapses.

In normal early embryonic life, MeCP2 is expressed in two developmentally important regions: the medulla and the thalamus. In normal brain development, the neurons of the medulla express critical trophic catecholamines (noradrenaline and serotonin)⁷⁴ and send nonspecific afferents to the developing cortex, where they influence migration and organization of neuronal systems. MeCP2 deficiency in brainstem neurons, subplate, and Cajal-Retzius neurons (and all neurons required for critical stages of development) would alter the efficacy of these neurons by interfering with their maturation, causing a reduced production of trophic factors,⁷⁴ receptors,⁷⁵ early response genes,⁷⁶ and structural proteins⁷⁷ and disorganization of cortical columns,⁵¹ dendrite growth, and synapse formation.⁴⁸

Studies of MeCP2 in the olfactory epithelium of mice have shown that MeCP2 is expressed prior to synapse formation and that interfering with synapse formation reduces the amount of MeCP2 expression.^{68,70} LaSalle's model suggests that in the normal maturation of neurons, the neurons that express a low level of MeCP2

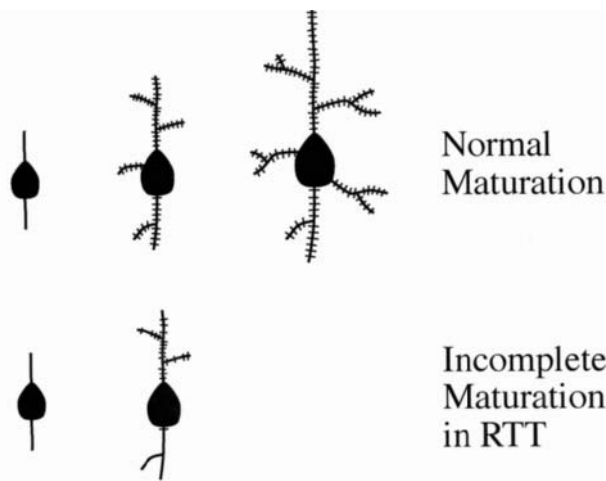


Figure 3. Normal maturation of the neuron, with a gradual increase in size, in dendritic branching, and in spines.

are modified by a non-cell-autonomous mechanism (possibly by synaptic stimuli from surrounding neurons) to express MeCP2 at a high level. Thus, through cell-autonomous and non-cell-autonomous regulation, the availability of MeCP2 in individual neurons will influence its role in the genetic regulation of maturation. In the Rett syndrome brain, with fewer synapses, the expression of MeCP2 is less than optimum. This direct role of MeCP2 in response to neuronal activity has been suggested by two other studies. Kaufmann and colleagues' identification of *MECP2* in the post-synaptic compartment of neurons places it in a site where it is available to respond to neuronal activity.⁷⁸ Chen and colleagues also demonstrated that calcium entry into the cell causes phosphorylation of MeCP2 and releases its inhibitory role, enabling the transcription of brain-derived neurotrophic factor.⁷⁹

The abnormalities that we have observed in the anatomy, chemistry, and clinical manifestations of Rett syndrome all suggest that *MECP2* is essential for neuronal maturation. The signal for the initial expression of *MECP2* in a neuronal precursor cell can be regulated by a cell-autonomous mechanism. Its increasing expression in fully mature neurons can then be regulated in part by external factors, such as synaptic input. Proteomic studies of olfactory epithelium at the age at which synaptogenesis is taking place demonstrate that *MECP2* deficiency alters some of the proteins required for neuronal outgrowth, path finding, and cell connections. It is also required for proteins involved in mitochondrial activity and stress management.⁸⁰ Thus, *MECP2* appears to be required for both the structural and the functional maturity of neurons (Figure 3). To promote neuronal maturation in children with Rett syndrome, it will be necessary now to determine how MeCP2 deficiency can be treated.

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Original Article

Dissecting *MECP2* Function in the Central Nervous System

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ABSTRACT

Rett syndrome is a neurodevelopmental disorder and an important cause of mental retardation and autistic behavior in girls and in a small group of boys. In 1999, mutation of the methyl-CpG binding protein 2 (*MECP2*) gene encoding a transcriptional repressor on the X chromosome was found to cause Rett syndrome. Since this discovery, significant research has focused on the elucidation of its specific role in the central nervous system. Recent studies revealed that *MECP2* is expressed in more differentiated neurons rather than in less differentiated neuroblasts and that *MECP2* is involved in the maturation and maintenance of neurons, including dendritic arborization and axonal projections, rather than in early cell fate decisions in the mammalian brain. In this review, we summarize recent findings regarding regional, temporal, and cell type-specific *MECP2* expression in the central nervous system; neurobiologic abnormalities in *MECP2*-mutant mice; and *MECP2* target genes in the central nervous system. (*J Child Neurol* 2005;20:753–759).

Rett syndrome, a neurodevelopmental disorder, is the second most common cause of mental retardation in girls.^{1–6} Rett syndrome is seen almost exclusively in female individuals, with a prevalence rate of 1 in 10,000 to 15,000.^{1–6} Recently, it has also been identified in a small group of boys.⁷ These children have an apparently normal development until 6 to 18 months, after which they undergo

a period of rapid regression, with loss of purposeful hand use, deceleration of head growth, and onset of repetitive, stereotyped hand movements. They develop irregular breathing, ataxia, and seizure. Patients with Rett syndrome typically survive into their forties and need substantial assistance from family members or require institutional care.